Evaluation and Comparison Under Field Conditions of the Stress Response Induced in Horses When Administered Endoparasiticides in Tablet or Paste Formulations*

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CLINICAL RELEVANCE

Administering equine anthelmintics in paste formulations has been integral to the management of equine parasites. The objective of this clinical trial was to assess whether a new palatable tablet presentation reduces stress in the horse when administering an anthelmintic. Horses (n=122) were randomly allocated into three homogenous groups: Group 1: tablets (Equimax® Tabs; Virbac); Group 2: paste A (Equimax® Gel; Virbac); and Group 3: paste B (Equest® Pramox; Fort Dodge). Stress response to deworming was assessed by monitoring heart rates (Horse Heart Rate Monitors, Polar Equine) before, during and after the administration of the allocated anthelmintic. In addition, eight scored behavioural reactions indicative of stress were assessed. The increase in heart rate was significantly less ($p<0.0001$) in Group 1 in comparison to Groups 2 and 3. Approximately 40% of Group 1 presented no behavioural reaction. More than 70% and 90% of the horses showed at least one reaction in Groups 2 and 3, respectively. Flehmen and pinning ears back reactions were significantly less frequent in Group 1 than in the paste groups ($p=0.0013$ and $p=0.0277$, respectively). According to the parameters monitored, this study demonstrates that the administration of an equine endoparasiticide in palatable tablet formulation induces less stress compared to a paste formulation.

INTRODUCTION

Management and control of internal parasites is an integral part of equine care and is responsible for the overall health of the horse. The timing, rotation, and product selection of an anthelmintic contribute to the frequency and effectiveness of a de-worming program. The choice of an equine de-wormer is generally based on its spectrum of activity and its safety. However, its formulation is another important parameter which should also be taken into account. Indeed, different formulations are currently available. The pastes are the most commonly used in the field. Nevertheless it is a well recognised fact that de-worming with certain pastes can be difficult and not well accepted by some horses. Furthermore, the horse may spit out some of the paste administered. Pellets, granules and some liquid suspensions are designed to be fed mixed with grain. However, some horses will not eat grain with the presence of a de-wormer product in it, or will not ingest the entire dose at once. Powder formulations are usually available only to veterinarians and are designed to be reconstituted and used as a liquid. These liquid suspensions are traditionally administered by the veterinarian using a stomach tube.[1]

The possibility of horses not taking the entire dose of an anthelmintic at once can lead to a problem of under-dosing, and the emergence of resistance.[1] The resistance of Cyathostominae spp. to benzimidazoles and pyrantel is well recognised, and has recently been suspected with regards to macrocyclic lactones.[2,3] As under-dosage is known to be a risk factor for the development of resistance,[4] it is important to administer macrocyclic lactones at the correct dose rates.

If anthelmintics are spontaneously taken by the horse, the use of palatable tablets seems to be an interesting alternative to enhance the ease of administration and to administer the appropriate dosage. The administration of a de-wormer without restraint and stress should improve compliance and the efficacy of de-worming programs, as well as contribute to the animal's welfare.

The main objective of this multi-center, controlled and randomised clinical pilot study was to assess under field conditions (i.e. representative of normal use of the products in non-experimental animals) the stress induced in horses when administered tablets of a new endoparasiticide (Equimax® Tabs) compared to oral pastes both with same indication and same target species: Equimax® Paste and Equest® Pramox. It was not the purpose of this study to evaluate the product acceptance or the palatability of tablets.

MATERIALS AND METHODS

Animals

The study was carried out in two European sites in France and in the United Kingdom in the spirit of the principles of Good Clinical Practice (VICH GL9 adopted by CVMP in June 2000; CVMP/VICH/595/98-FINAL). Healthy horses of various breeds, age, bodyweight and gender were enrolled. For safety reasons, only horses more than twelve months of age were included, even if label recommendations of investigational products would have allowed selecting younger animals. Horses known to be hypersensitive to active ingredients or excipients of test products, as well as pregnant and lactating mares were not included in order to respect the restrictions of use as stated by the product licences across all test products. All animals intended to be treated remained in the evaluation of the results. No other treatment was allowed during the study, and horses remained in their usual environment during study procedures. Only horses that were scheduled for de-worming at the time of the study were included. The animals were allocated to each treatment group according to a predefined order. The number was fixed to 40 per group (20 per country) in order to have a representative number of animals in each group. The persons responsible for the horses agreed that they would be included in the study and signed an informed consent form.

Treatment

Treatment was administered by the usual handler or horse rider, and according to individual bodyweight. Horses were individually weighed prior to treatment (but not on the day of treatment) using calibrated scales, or the weight was estimated on the day of treatment when scales were not available. At each clinical site, horses selected to participate in the study were randomly allocated into three groups according to a randomisation list (table of permutations at random with three elements). The groups were homogeneous in terms of body weight, age and gender of animals included and group size. The groups were also balanced between the countries involved in the study. Horses received a single oral administration of: one tablet (Equimax® Tabs; Virbac) per 100kg bodyweight, equivalent to 200 µg/kg ivermectin and 1.5 mg/kg praziquantel in Group 1; one graduation of paste A (Equimax® Gel; Virbac) per 100kg bodyweight, equivalent to 200 µg/kg ivermectin and 1.5 mg/kg praziquantel in Group 2; or one graduation of paste B (Equest® Pramox; Fort Dodge) per 100kg bodyweight, equivalent to 400 µg/kg moxidectin and 2.5 mg/kg praziquantel in Group 3. For all three products, the recommended dose rates for de-worming horses were respected. This study being not an efficacy study it was acceptable to have the body weight estimated in some cases since precise body weight has no link to behavioural response.

Administration

A standardised method of administration was performed for each product. Tablets were presented to the horse in the palm of the handler's hand until the complete dose had been administered. If the horse did not spontaneously accept the tablets, they were combined with a treat the horse was used to being given (e.g. apple, carrot, sugar etc.). If the horse still refused to accept the tablets, they were placed in the feed bin or bucket and mixed with a small amount of usual feed. In this case, the handler ensured that the full dose was ingested. Pastes were administered according to respective label directions according to the usual and well established method by experienced horse handlers. The syringe was adjusted to the bodyweight by locking the ring at the appropriate place on the plunger.

Parameters monitored

The following parameters were monitored by the same veterinary surgeon for all horses included in the trial.

- Heart rate

Heart rate was measured before, during and after the treatment to assess the stress induced by the administration of each product. For a given horse, the same conditions were maintained throughout the measurement period. In particular, all individuals were present at all stages of the administration, in order to maintain consistency between horses.

The heart rate monitors (Horse Heart Rate Monitors (HHRM) Polar Equine, Biarritz, France) were placed on horses before recording any data. Once the horse had settled down (i.e. when heart rate was stable; approximately 1-3 minutes after the monitors were placed) the heart rate was recorded three times, approximately 15 seconds apart, at each of the three following time periods: before (i.e. when the horse had settled down; prior the beginning of administration and before showing any dewormer), during (i.e. when the horse received the product = within 1 minute after the administration started) and after (i.e. after the end of treatment, when the horse returned to normal behaviour and had fully ingested the products = approximately 2 minutes after the administration) the administration of the allocated anthelmintic product.

Beat per minute values were directly displayed on the screen of the device and read by the investigator. The average of the three readings was calculated for each time period. Moreover, the HHRM electronically recorded the horse's heart rate which was downloaded onto a computer afterwards.

- Behavioural reactions

Since behavioural reactions are subjective assessments, no scoring was implemented but only "presence/absence". The person administering the product and the investigator evaluated the behavioural reactions together.

The presence or absence of each of the eight following behaviour reactions indicative of stress was individually evaluated by the investigator: attempt to flee, defence, pinning ears back, raising / turning head, tossing and shaking head, defaecating, salivation, and flehmen. Selection of the parameters was based on literature[5-10] and knowledge of horse behaviour.

Statistical analysis

The statistical analysis was performed using "Statview" software 5.0 (SAS Institute Inc, USA). Descriptive statistics of age and bodyweight were given for each group. Heart rate, a continuous variable, was evaluated by a repeated measure, two-way analysis of variance taking into account the effect of treatment and the effect of time. In case of significant time*treatment interaction, a comparison of the treatment groups was made for each time. A statistical analysis of the different examination values was carried out for each time using the ANOVA (comparison between treatment groups and, if significant, pair-wise comparisons) followed by a Scheffé post hoc comparison. The alteration in heart rate compared to baseline value was calculated at each time point. Behavioural reactions were summarized per group. The numbers of behavioural reactions were compared between treatment groups using the non parametric test of Kruskall Wallis followed by a pair-wise comparison (Mann-Whitney). Significance was stated if $P < 0.05$.

RESULTS

Animals Included

One hundred twenty-two horses (i.e. 61 horses in each study site) were enrolled in the study. This included 83 (68%) males (5 stallions and 78 neutered males) and 39 (32%) females. The following different breeds were represented: French Saddle, Lusitanian, Andalusian, Warmblood, Thoroughbred, Welsh, Cob, Exmoor, Shetland and crossbred. On the day of treatment, the mean age of the animals was 11.0 ± 5.2 years (with a minimum of 1 year and a maximum of 23 years) and their mean bodyweight was 562.5 ± 108.2 kg (with a minimum of 120 kg and a maximum of 700 kg). The majority of horses were kept day and night in individual box stalls (91 horses, 74.6%); some were stabled overnight (14, 11.5%), and the remainder kept in paddocks (13 horses, 10.7%), or on pasture (4 horses, 3.3 %). Animal baseline characteristics are fully presented per treatment group in Table 1, statistical analyses indicated that the three treatment groups were comparable with respect to gender, age and bodyweight on treatment day ($p > 0.05$).

Stress assessment

- Monitoring of heart rate

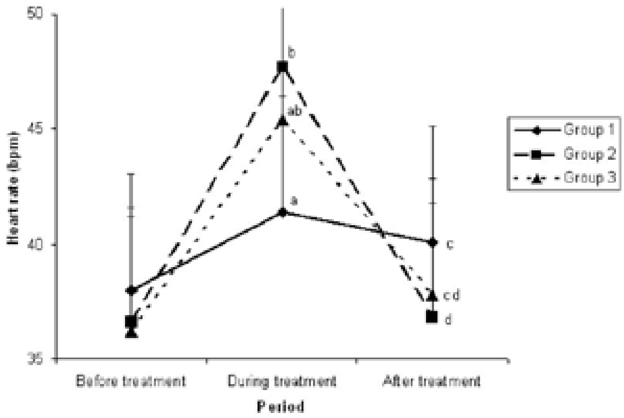
Mean heart rates measured in each treatment group at the different time points are shown in Fig. 1. The values were similar in all groups before treatment. Heart rates increased during treatment in all groups however the increase was significantly less with the tablets compared to the pastes ($P < 0.0001$). The mean increase in

***Table 1** Description of animal baseline characteristics in each treatment group*

	Group 1	Group 2	Group 3	Total
Number of animals	41	41	40	122
Age* (years)	11.4 ± 5.5	11.1 ± 5.2	10.5 ± 5.0	11.0 ± 5.2
Bodyweight* (kg)	542.1 ± 118.2	584.1 ± 103.7	561.2 ± 100.1	562.5 ± 108.2
Gender	14 females, 3 males, 24 neutered males	13 females, 1 male, 27 neutered males	12 females, 1 male, 27 neutered males	39 females, 5 males, 78 neutered males

** mean ± standard deviation*

Fig. 1 *Mean heart rates measured before, during and after each treatment*



Error bars indicate 95% confidence intervals.
a,b Means with no letters in common differ (p = 0.0140)
c,d Means with no letters in common differ (p = 0.0235)

heart rate during treatment compared to pre-treatment values was 3.2 ± 6.3 bpm (beats per minute) in Group 1 compared to 11.1 ± 11.2 bpm and 9.2 ± 10.2 bpm in Groups 2 and 3, respectively. This increase was statistically lower in Group 1 compared to the other groups (P = 0.008). Pair-wise comparisons for the variation in mean heart rate between "before" and "during" treatment confirmed that variations observed in Group 1 significantly differed from those registered in the two paste groups (Fig. 2).

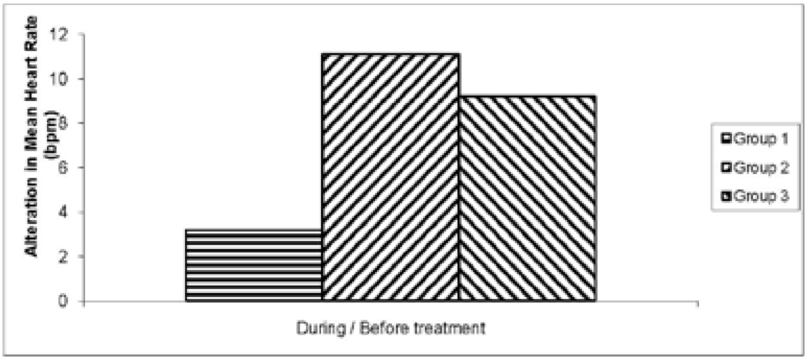
- Behavioural reactions

Behavioural reactions are summarized per group in Fig. 3 and 4. The mean number of behavioural reactions per horse was 1.3 ± 1.5 in Group 1, 2.0 ± 1.7 in Group 2, and 2.1 ± 1.3 in Group 3. Approximately 40% of the animals presented no reaction in Group 1, whereas more than 70% and 90% of the horses showed at least one reaction in Groups 2 and 3, respectively. Using the non parametric test for categorical values, the difference was significant between groups (Kruskall Wallis, P = 0.032); Equimax® tabs (group 1) was significantly different from Equest Pramox® (group 3) (Mann-Whitney, P = 0.0084). The most frequently observed reactions during treatment were raising/turning head, pinning ears back, tossing and shaking head, and flehmen. Defence reaction and attempt to flee were less frequent, and defaecating and salivation were occasional. Considering each parameter individually, pinning ears back and flehmen reactions were significantly less frequent in Group 1 in comparison to Groups 2 and 3 (P = 0.0277 and P = 0.0013, respectively). This was confirmed by pair-wise comparisons (P = 0.0156 between Groups 1 and 2, P = 0.0156 between Groups 1 and 3 for pinning ears back, and P = 0.0017 between Groups 1 and 2, P = 0.0003 between Groups 1 and 3 for flehmen).

DISCUSSION

This study was not performed to assess palatability. The monitored parameters were focused on the assessment of the stress response induced by anthelmintic administration, based on the measurement of heart rate and behavioural observations. The choice of these non-invasive parameters as indicators of stress could be complemented by other examinations, as previous works have indicated that other parameters, such as PCV,[5] and plasma levels of cortisol,[11,12] cathecholamines,[5] glucose,[5] lactate concentrations[11] and β-endorphin[12] may also give an indication of stress in horses, However the procedure of blood sampling was consid-

Fig. 2 *Change in mean heart rate between baseline and during treatment*



a,b Means with no letters in common differ (p = 0.0002 between Groups 1 and 2, and p = 0.0025 between Groups 1 and 3)

Fig. 3 *Percentages of horses per treatment group exhibiting between 0 and 6 behavioural reactions*

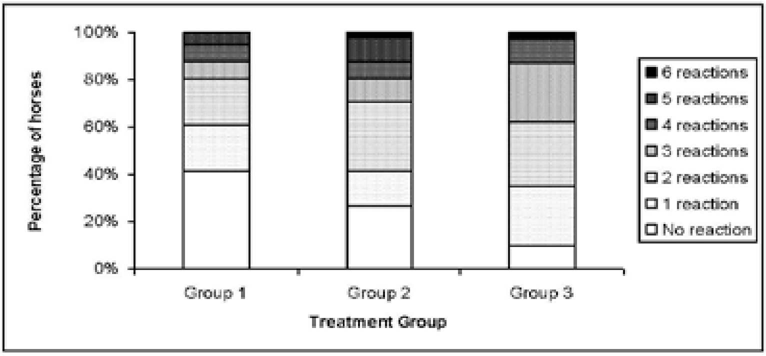
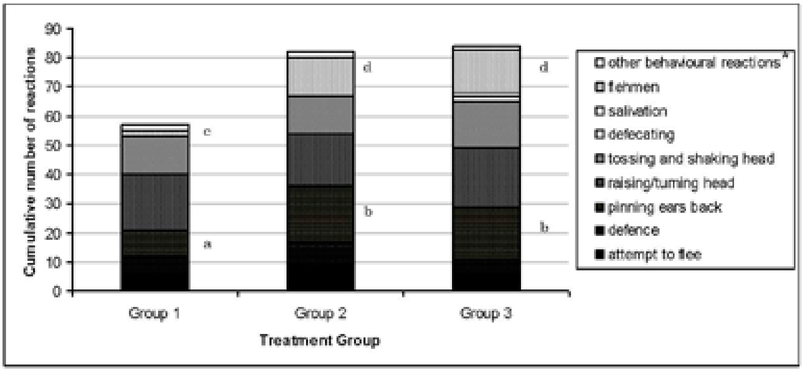


Fig. 4 *Distribution of horses per treatment group exhibiting each of the monitored behavioural reactions*



** Other behavioural reactions included an increase of respiratory rate in one horse in Group 3, manifestation of slight displeasure in one horse in Group 2, kicking with front legs in two horses, one in Group 1 and one in Group 2, and licking the wall in one horse in Group 1.*
a,b Means with no letters in common differ (p = 0.0277)

c,d Means with no letters in common differ (p = 0.0013) ered too invasive in the scope of this study. The treatments were not blinded because the investigators have to be present while the products were administered. Any movement of the staff during the observation period had to be avoided to limit the impact on the stress response. The behavioural reactions - subjective parameters - were evaluated by two persons to limit possible bias. However, the main parameter was the heart rate which was measured using a monitoring device. No untreated control group was included since each horse was its own control (comparison to before treatment).

Stress may be defined as a homeostatic, physiological, and behavioural response detectable in an animal as a result of interactions with environmental stressors.[6,13,14] When stress is induced, two neuroendocrinological axes, the sympatho-adrenomedullary axis and the hypothalamo-pituitary-adrenocortical system, are activated.[7,11] The increase of heart rate is a well known effect of the activation of the sympatho-adrenomedullary axis.[7,15] Some studies demonstrated that changes in heart rate indicate an emotional response to a short-term stressor.[16] Therefore heart rate measurement seems to be accurate to evaluate the stress response induced by the administration of a medication.

The hypothalamic-pituitary-adrenal axis and the production of cortisol is the most intensively studied stress pathway in horses,[5,15] and salivary cortisol can be used as a measure of stress. However within any study, strict control would need to be placed on individual variation, diurnal patterns and environmental effects which would be difficult to achieve with any accuracy within the presented study. Because many factors influence the plasma cortisol level in horses, some authors assert that it is not a very reliable variable to measure stress,[5] and controversial results of stress-related blood parameters have been observed.[8] This may be related to methodology as, for example, the blood sampling procedure itself may cause significant stress to an animal. Furthermore, the basal level of physiological parameters may differ between individuals depending on breed, age, gender, management and previous experience. Often, the inter-individual variation of such parameters is higher than the intra-individual difference between basal and experimental levels. This makes inter-individual comparisons difficult and complicates the interpretation of results.[8]

Currently, the assessment of stress responses is difficult in horses as well as in other species, as different physiological systems are involved (e.g. neuroendocrine and cardiovascular), which are manifested in the behavioural stress response.[9,17] A positive correlation between heart rate and plasma cortisol concentrations has been observed, whereby both increased during stimulation.[6,11,18] In addition, a correlation of behaviour to both heart rate and plasma cortisol has been described in horses in stressful situations.[11,18,19] However, changes in stress indicators may not be simultaneous. For instance, modifications of cardiac function may occur in an anticipatory manner prior to the expression of any alterations in behaviour, and it is not uncommon to see tachycardia several seconds before the emergence of a behavioural flight in horses.[20] On the other hand, a cardiac response may also persist beyond the expression of the specific behavioural event that it was initially associated with.[20]

The specificity of a single stress parameter for a certain stimulus is poor, and recording different parameters simultaneously is therefore necessary.[7] Horses express desires, intentions, and emotional states through their posture.[21] Various signals are used by horses to communicate with other animals depending on the situation or state of arousal.[21] In addition to heart rate monitoring, eight behaviour reactions were observed in the present study. Most of these specific equine behaviours were considered in previous studies to indicate stress, irritation, or frustration.[22,23] They included having the ears pinned back, raising the head, turning the head to left to right, tossing the head, shaking the head, holding the head down, and defecating.[22] Head movements and ear position have been effectively described as the best indicators of horse emotional states, [7,8,21,24,25] and are relatively accurate predictors of equine agitation related to displeasure, aggression, defence or irritation.[23,24,26] In this study, much of the difference in the behavioural responses relates to movements of the head. Administration of tablets resulted in less movement of the head compared to horses in which someone was forcibly administering a paste de-wormer. Defaecation can occur as a result of stimulation or arousal and therefore could indicate tenseness and fear.[27,28] In horses, the flehmen reaction may signify stress or anxiety.[29]

CONCLUSIONS

Parameters monitored in the present study were proven to be reliable indicators of stress as described in the literature. The results showed that the administration of an equine endoparasiticide in tablet formulation induced less stress compared to a paste formulation, evidenced by parameters such as heart rate, incidence of pinning ears back and flehmen. Minimizing stress in the horse, as well as in the horse owner, may contribute to improved compliance and thus the efficacy of the de-worming treatment, and contributes to the animal's welfare.[14,30]

REFERENCES

1. Grosjean H: Epidémiologie des parasitoses intestinales équines : Etude de quatre établissements du nord de la Loire. Mise au point d'un plan de vermifugation. *Thèse de Doctorat Vétérinaire, Alfort*. 2003.
2. Trawford AF, Burden F, Hodgkinson J: Suspected moxidectin resistance in cyathostomes in two donkey herds at the donkey sanctuary, Proceedings of the 20th International Conference of the World Association for the Advancement of Veterinary Parasitology 196, 2005.
3. Von Samson-Himmelstjerna G, Fritzen B, Demeler J, Schumann S, Rohn K, Schnieder T, Epe C: Cases of reduced cyathostomine egg reappearance period and failure of Parascaris equorum egg count reduction following ivermectin treatment as well as survey on pyrantel efficacy on German horse farms. *Vet Parasitol* 144: 74-80, 2007.
4. Sangster NC: Pharmacology of anthelmintics resistance in cyathostomes: will it occur with the avermectin/milbemycins? *Vet Parasitol* 85: 189-204, 1999
5. Sloet van Oldruitenborgh-Oosterbaan MM, Blok MB, Begeman L, Kamphuis MCD, Lameris MC, Spierenburg AJ, Lashley MJJO: Workload and stress in horses: comparison in horses ridden deep and round ('rollkur') with a draw rein and horses ridden in a natural frame with only light rein contact. Tijdschr Diergeneeskd 131: 152-157, 2006.
6. Stephens DB: Stress and its measurement in domestic animals. A review of behavioural and physiological studies under field and laboratory situations. *Adv Vet Sci Comp Med* 24: 179-210, 1980.
7. Rietmann TR, Stuart AEA, Bernasconi P, Stauffacher M, Auer JA, Weishaupt MA: Assessment of mental stress in warmblood horses: heart rate variability in comparison to heart rate and selected behavioural parameters. *Appl Anim Behav Sci* 88: 121-136, 2004.
8. Bachmann I, Bernasconi P, Herrmann R, Weishaupt MA, Stauffacher M: Behavioural and physiological responses to an acute stressor in crib-biting and control horses. *Applied Animal Behaviour Science* 82: 297-311, 2003.
9. Bohnet W: Expressive behaviour to assess the emotional states in horses. Dtsch. Tierarzt Wochenschr 114: 91-97, 2007.
10. Nagy K., Bodo G., Bardos G., Harnos A., Kabai P. - The effect of a feeding stress-test on the behaviour and heart rate variability of control and crib-biting horses (with or without inhibition). *Applied Animal Behaviour Science* 121: 140–147, 2009.
11. Covalesky ME, Russoniello CR, Malinowski K: Effects of show-jumping performance stress on plasma cortisol and lactate concentrations and heart rate behavior in horses. *J Equine Vet Sci* 12: 244-251, 1992.
12. McGreevy P, Nicol C: Physiological and behavioral consequences associated with short-term prevention of crib-biting in horses. *Physiol Behav* 65: 15-23, 1998.
13. McEwen BS: Stress and hippocampal plasticity. *Annu Rev Neurosci* 22: 105-122, 1999.
14. Moberg GP, Mench JA: The biology of animal stress. Basic principles and implications for animal welfare. Wallingford, Oxon, CABI International, 2000, pp377.
15. Friend TH: Stress: what is it and how can it be quantified? *Int J Study Anim Probl* 1: 366-74, 1980.
16. Minero M, Canali E, Ferrante V, Verga M, Odberg FO : Heart rate and behavioural responses of crib-biting horses to two acute stressors. *Vet Rec* 145: 430-433, 1999.
17. Herd JA: Cardiovascular response to stress. *Physiol Rev* 71: 305-330, 1991.
18. Dentzer R, Moremede P: Stress in farm animals: a need for re-evaluation. *J Anim Sci* 57: 6-18, 1983.
19. Mench JA, Van Tienhoven A: Farm animal welfare. *Amer Scientist* 74: 598-603, 1986.
20. Von Borell E, Langbein J, Despres G, Hansen S, Leterrier C, Marchant-Forde J, Marchant-Forde R, Minero M, Mohr E, Prunier A, Valance D, Veissier

I: Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals - a review. *Physiol Behav* 92: 293-316, 2007.

21. Weeks J, Beck AM: Equine agitation behaviors. *Equine Pract* 18: 23-24, 1996.
22. Kaiser L, Heleski CR, Siegford J, Smith KA: Stress-related behaviors among horses used in a therapeutic riding program. JAVMA 228: 39-45, 2006.
23. McDonnell SA: A practical field guide to horse behaviour: the equid ethogram. Lexington, Ky, The Blood-Horse Inc, 2003, pp 33-46, 74, 92-95, 110-112, 1187.
24. Ainslie T, Ledbetter B: The body language of horses. New-York, Morrow, 1980.
25. Houpt KA: Domestic animal behavior for veterinarians and animal scientists. Ames, IA, Iowa State University Press, 1991.
26. Houpt KA, Eggleston A, Kunkle K, Houpt TR: Effect of water restriction on equine behaviour and physiology. *Equine Vet J* 32: 341-344, 2000.
27. Schäfer M: Pferd. In: Sambraus HH (Ed) Nutztierethologie. Das verhalten landwirtschaftlicher nutztiere-Eine angewandte verhaltenskunde für die Praxis. Berlin, Paul Parey, 1978, pp 214-248.
28. Waring GH: Horse behaviour, 2nd edition. Norwich, NY, Noyes Publication, William Andrew Publishing, 2003.
29. Crowell-Davis SL: Flehmen: "What's My Horse Doing with His Lip?" *Equine Review* HW 41, 2008.
30. Von-Borell E, Veissier I: Special section: Stress and welfare in farm animals. *Physiol Behav* 92: 291-433, 2007.